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Study on pollen viability and stigma receptivity throughout the flowering period in the selected taxa of the Gesneriaceae family

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ABSTRACT

Plants in the Gesneriaceae family are appreciated for their decorative leaves and flowers, ease of cultivation, and shade tolerance. Sexual hybridisation has long been carried out for producing novel hybrids. However, scientific knowledge is lacking on the correct timing of pollination in this family. This study was conducted to elucidate the optimal timing for pollination by screening pollen viability and stigma receptivity throughout the flowering period in eight gesneriad taxa. Pollen viability was evaluated by the *in vitro* germination test and stigma receptivity was based on stigma morphology and pistil length. The flowering duration varied from 10 days in *Alsobia* to 18 days in *Streptocarpus*. While the pollens of *Episcia, Kohleria, Saintpaulia, Sinningia,* and *Smithiantha* had totally lost viability 2–5 days before the flowers withered, a slightly contrasting situation was observed in the cases of *Alsobia, Deinostigma,* and *Streptocarpus*, where a small portion of pollen grains remained viable towards the end of the flowering period. The highest pollen germination rate was recorded from 1 day in *Alsobia*, *Episcia*, and *Kohleria*, to 9 days in *Deinostigma*. The reduction in pollen germination was rapid in *Alsobia*, *Saintpaulia*, and *Smithiantha*, and moderate in the remaining taxa. The greatest pollen tube growth occurred at 1–3 days after flower opening and decreased rapidly in all the taxa tested. The longest pistil of 2.03–3.50 cm was observed at 3–8 days of anthesis depending on the plant tested. The findings in this study suggest that higher pollination success may be achieved using pollen grains of newly opened flowers and stigmas of mature flowers in this family.

Keywords: gesneriad, pistil length, pollen germination, pollen tube length, pollination timing

INTRODUCTION

Gesneriaceae is an angiosperm family of the order Lamiales with large-scale species richness. There are roughly 160 genera and more than 3,300 species in the family, including perennial herbs, shrubs, and small trees, which are mostly distributed in tropical and subtropical zones around the globe (Möller and Clark, 2013; Weber et al., 2013). Many are of economic importance and are frequently used for potted flowers or in landscaping. Breeding of gesneriads has long been carried out to meet the market demand for novel hybrids, and was mostly achieved via sexual hybridisation.

Pollen grains are sexual reproductive units in plants and bearers of male genetic material (Halbritter et al., 2018). Understanding the variables that influence pollen

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viability is critical in genetic breeding programmes (Dane et al., 2004). Pollen viability encompasses several features of pollen performance, including germination, pollen tube elongation, and fertilisation abilities (Dafni and Firmage, 2000). Staining, *in vitro* germination, and seed set as well as semi-*in situ* germination on the excised stigma are common methods for determining pollen viability. Although staining techniques can distinguish between abortive and non-aborted fresh pollen, they frequently fail to distinguish between different levels of pollen viability (Ge et al., 2011). In the last two methods, contrasting dye is used to detect pollen tube growth towards or on stigmas, and the results are thought to provide the most precise seed set estimates (Dionne and Spicer, 1958). Nevertheless, incompatibilities, post-fertilisation obstacles, and restricted measurability may limit the accuracy of these assays (Dafni and Firmage, 2000). *In vitro* pollen germination test has been adopted to evaluate pollen viability in many plants by tracking the percentage of pollen germination and the length of the pollen tube over time (Shivanna and Mohan Ram, 1993). Pollen grains that fail to germinate frequently have weak pollen tube growth and are therefore ineffective during sexual fertilisation (Lin et al., 2017). Different medium formulations are required for different plant species to germinate their pollen grains (Baloch et al., 2001). Among the medium supplements, sucrose, boric acid, and calcium nitrate are among the most commonly used in various studies (Tushabe and Rosbakh, 2021).

Stigma is a glandular organ whose key functions are to receive, recognise, and provide the pollen grains with germination substrate (Hiscock and Allen, 2008). At various phases of flower development, stigma receptivity determines the rate of pollination success (Galen et al., 1987). Stigma receptivity can be determined using morphology, seed set ability, and cytochemical testing for the existence of enzyme activity (Dumas et al., 1984). Stigma characterisation has been extensively illustrated, both anatomically and biochemically (Heslop-Harrison and Shivanna, 1977). According to the pattern of secretion, two types of stigmas are recognised in histology: wet and dry stigmas (Heslop-Harrison and Shivanna, 1977). The wet stigma's secretion is exposed to the environment, but the dry stigma contains a proteinaceous pellicle overlying a discontinuous cuticle that keeps secretions within the stigma (Lersten, 2004).

Research on pollination ecology and pollen morphology has been conducted in some gesneriads over the last 70 years, but mostly focussed on native and endangered species (Erdtman, 1952; Melhelm and Mauro, 1973; Fritze and Williams, 1988; Luegmayr, 1993; Gao et al., 2006; Lazarevic et al., 2013). Aside from the cited articles, pollen studies in commercially important gesneriads are scarce. Therefore, this study aimed to provide scientific information on pollen viability and stigma receptivity throughout anthesis in eight popular gesneriad species in order to determine the best pollination timing for this family.

MATERIALS AND METHODS

Plant material

Eight selective taxa of the *Gesneriaceae* family were used in this study, which included two species namely *Deinostigma eberhardtii* (Pellegr.) D.J. Middleton & H.J. Atkins and *Streptocarpus saxorum* Engl., and six horticultural hybrids such as *Alsobia* 'Cygnet', *Episcia* 'Thad's Yellow Bird', *Kohleria* 'Brimstone', *Saintpaulia* 'Lyon's Party Parasol', *Sinningia* 'HCY's Peach Fragrance', and *Smithiantha* 'An's Hanabi' (Figure 1). Each species or hybrid was propagated to increase the number of individuals for the study. Plants of *Alsobia* 'Cygnet', *Deinostigma eberhardtii*, *Episcia* 'Thad's Yellow Bird', *Saintpaulia* 'Lyon's Party Parasol', and *Streptocarpus saxorum* were propagated by stem or leaf cuttings to yield ca. 10 individuals, whereas plants of *Kohleria* 'Brimstone', *Sinningia* 'HCY's Peach Fragrance', and *Smithiantha* 'An's Hanabi' were propagated by rhizomes or tubers to produce three to six individuals. All the plants were grown in a 2:1:1 substrate combination of peat moss, perlite, and vermiculite inside 5″ pots. Each month, the plants were fertilised with Hi-Control fertiliser (14N-11P-13K). The greenhouse was maintained at 26 ± 4 °C and ≈75–80% relative humidity without supplemental lighting. Water was applied directly to the substrate twice a week without wetting the leaves of the plants.

Evaluation of pollen viability

A preliminary trial was conducted on the *in vitro* pollen germination of *Smithiantha* 'An's Hanabi' to evaluate the requirement of sucrose, boric acid (H_3BO_3), and calcium nitrate [$Ca(NO_3)_2 \cdot 4H_2O$] for pollen germination. A total of 12 media containing 10%, 20%, or 30% sucrose, 50 mg · L^{-1} or 100 mg · L^{-1} boric acid, and 200 mg · L^{-1} or 400 mg · L^{-1} calcium nitrate were tested, together with the water agar (WG) medium devoid of sucrose, boric acid, and calcium nitrate as the control. Pollen grains were collected from three flowers of mixed ages and of different flowering individuals. The pollen grains were suspended in distilled water and 10 drops of 1 µL each of pollen suspension were inoculated on the surface of 10 mL pollen germination medium contained in a 6-cm Petri dish. The Petri dishes were incubated at 25 ± 2 °C in the dark for 24 h. Pollen germination and pollen tube growth were assessed under a light microscope with 10x magnification. The pollen grains were considered as germinated if the length of the pollen tube was equal to or greater than the grain diameter (Kakani et al., 2005). The numbers of germinated and non-germinated pollen grains were counted in 10 fields (one field per drop) in each Petri dish. The percentage of pollen germination was evaluated using Eq. (1) (Kakani et al., 2005). Pollen tube length was analysed with ImageJ software, version 1.52v (National Institutes of Health, Bethesda, MD, USA) and Java 1.8.0_112. The mean pollen tube length (in centimetres) was estimated as the average of

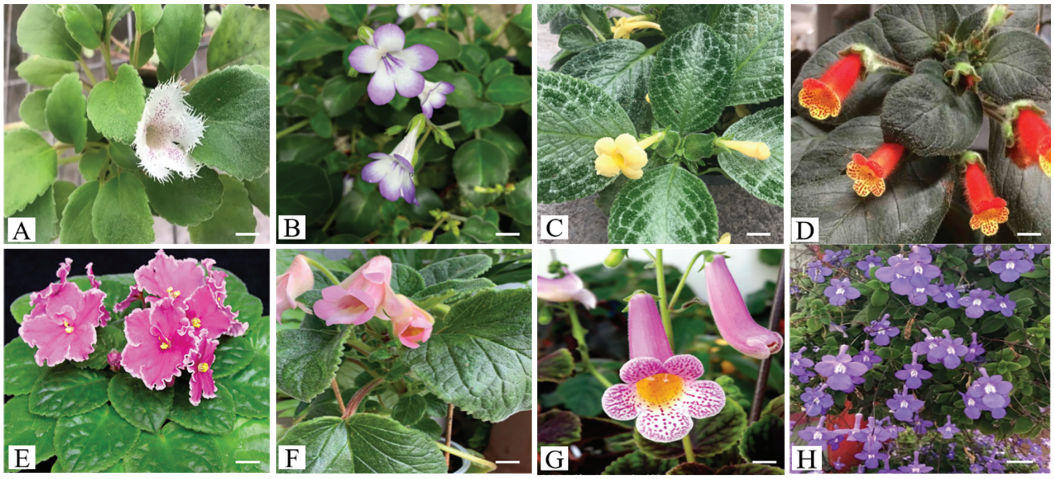

Figure 1. The eight gesneriad taxa used in this study. (A) *Alsobia* 'Cygnet'. (B) *Deinostigma eberhardtii*. (C) *Episcia* 'Thad's Yellow Bird'. (D) *Kohleria* 'Brimstone'. (E) *Saintpaulia* 'Lyon's Party Parasol'. (F) *Sinningia* 'HCY's Peach Fragrance'. (G) *Smithiantha* 'An's Hanabi'. (H) *Streptocarpus saxorum*. Bars = 1 cm.

30 randomly selected pollen tubes from each of the 10 fields and calculated using Eq. (2).

$$\text{Pollen germination (\%)} = \frac{\text{Germinated pollen grains per field}}{\text{Total pollen grains per field}} \times 100 \quad (1)$$

$$\text{Pollen tube length (cm)} = \frac{\text{Total length of 30 selected pollen tubes}}{30} \quad (2)$$

Subsequently, the *in vitro* pollen germination test was narrowed down to four germination media containing 10% sucrose, 50 mg · L^{-1} or 100 mg · L^{-1} boric acid, and 200 mg · L^{-1} or 400 mg · L^{-1} calcium nitrate, together with the control (CK) medium devoid of boric acid and calcium nitrate on eight gesneriad taxa.

Pollen viability was evaluated by determining pollen germination and pollen tube length of eight gesneriad taxa at each day of the flowering period. Pollen grains were inoculated on the optimised *in vitro* germination medium. The pollen germination percentages and pollen tube lengths were evaluated as described previously.

Evaluation of stigma receptivity

The morphology of the stigma was monitored daily and the day at which the stigma opened was recorded for the plant analysed. Evaluation of pistil length was conducted each day, from 7:00 a.m. to 10:00 a.m. Three flowers of each plant were recorded for the pistil length by using a ruler. The length of the pistil was measured from the top of stigma to the base of the ovary.

Experimental design and data analysis

All experiments were arranged in a completely randomised design with three replicates per treatment and/or taxon. All trials were conducted on the same day to eliminate variation caused by non-experimental treatment conditions. Graphs were made using Microsoft Excel software (Microsoft Corporation, Redmond, Washington, USA). Data means were subjected to one-way ANOVA for analysis and compared with Duncan's multiple range test using Statistical Package for Social Sciences (SPSS) for Windows, version 26 (International Business Machines Corporation, Armonk, New York, USA).

RESULTS

Evaluation of pollen viability

In the preliminary trial on pollen germination of *Smithiantha* 'An's Hanabi', it was found that the highest pollen germination rates were recorded on A1–A4 media containing 10% sucrose (54%–92%), followed by B1–B4 media containing 20% sucrose (10%–13%) and WG medium (15%) (Figure 2). None of the pollen grains germinated on C1–C4 media were enriched with 30% sucrose. The pollen tube lengths were also greater on A1–A4 media with 10% sucrose (1.47–1.64 cm) compared to B1–B4 media with 20% sucrose (0.59–0.62 cm) and WG medium (0.86 cm). Based on this, the *in vitro* pollen germination test was performed only on media containing 10% sucrose in the subsequent experiment involving eight gesneriad taxa.

When testing the eight gesneriad taxa, higher germination percentages were observed in A1 and A2 media than in A3 and A4 media for *D. eberhardtii*, *Episcia* 'Thad's Yellow Bird', *Kohleria* 'Brimstone', *Saintpaulia* 'Lyon's Party Parasol', *Sinningia* 'HCY's Peach Fragrance', and *Smithiantha* 'An's Hanabi' (Figure 3). In *Alsobia* 'Cygnet', the media A1, A2, and A3 produced higher pollen germination percentages compared to the medium A4. The pollen germination percentages were equivalent across all four media

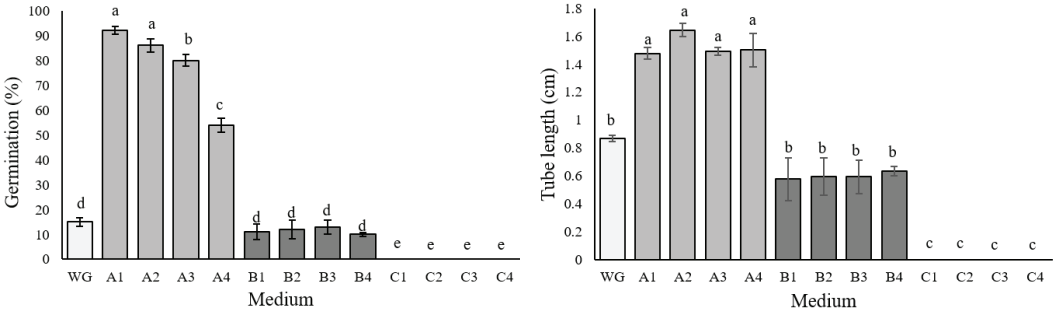

Figure 2. Pollen germination percentages and pollen tube lengths of *Smithiantha* 'An's Hanabi' in different germination media. Medium specification (WG: 0C-0B-0C; A1: 10S-50B-200C; A2: 10S-50B-400C; A3: 10S-100B-200C; A4: 10S-100B-400C; B1: 20S-50B-200C; B2: 20S-50B-400C; B3: 20S-100B-200C; B4: 20S-100B-400C; C1: 30S-50B-200C; C2: 30S-50B-400C; C3: 30S-100B-200C; C4: 30S-100B-400C; S: sucrose; B: boric acid; C: calcium nitrate). Means with identical lowercase letters are not significantly different (Duncan test, $*p < 0.05$). WG, water agar.

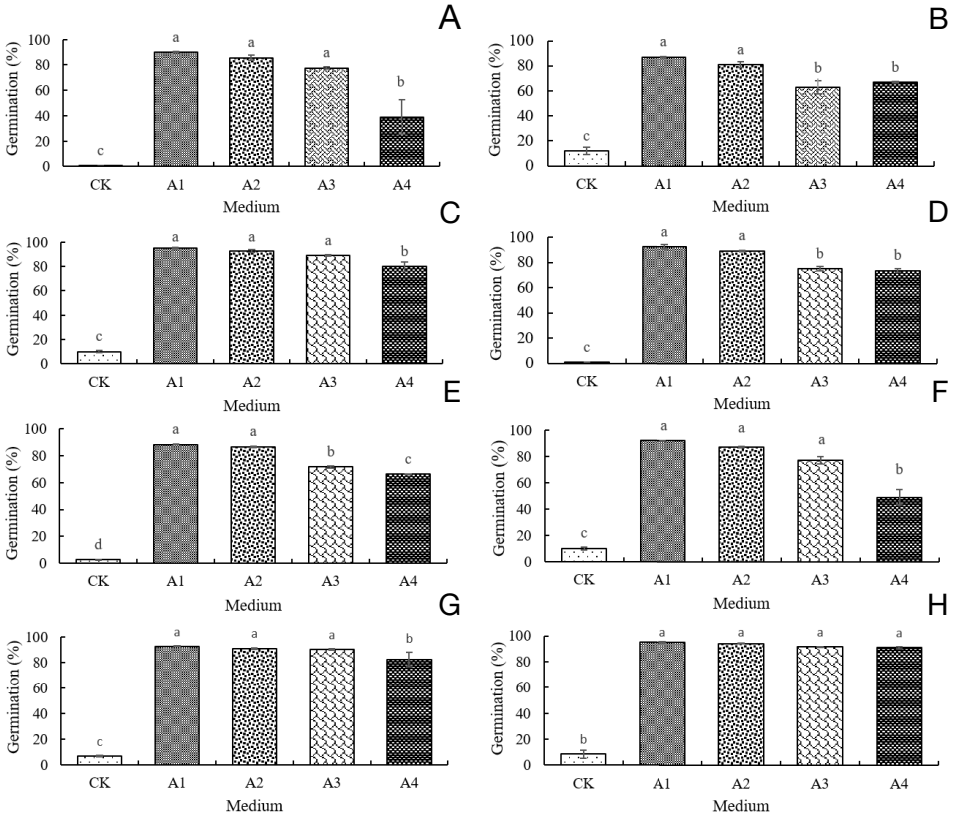

Figure 3. Pollen germination percentages of eight gesneriad taxa in different germination media. (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Saintpaulia* 'Lyon's Party Parasol', (F) *Sinningia* 'HCY's Peach Fragrance', (G) *Streptocarpus saxorum*, and (H) *Smithiantha* 'An's Hanabi'. Medium specification (CK: 10S-0B-0C; A1: 10S-50B-200C; A2: 10S-50B-400C; A3: 10S-100B-200C; A4: 10S-100B-400C; S: sucrose; B: boric acid; C: calcium nitrate). Means with identical lowercase letters are not significantly different (Duncan test, $*p < 0.05$).

in *S. saxorum*. Regarding pollen tube length, longer pollen tubes were found in the A1 medium than in the A2, A3, and A4 media for *Saintpaulia* 'Lyon's Party Parasol', *Sinningia* 'HCY's Peach Fragrance', and *Smithiantha* 'An's Hanabi' (Figure 4). In *D. eberhardtii* and *S. saxorum*, the A1 and A2 media produced longer pollen tubes than the A3 and A4 media. In *Alsobia* 'Cygnet' and *Kohleria* 'Brimstone', the media A1, A2, and A3 exhibited greater pollen tube lengths than the A4 medium. There was no significant difference in pollen tube lengths among the four media in *Episcia* 'Thad's Yellow Bird'. In all the plants tested, the medium CK yielded the lowest pollen germination percentages and pollen tube

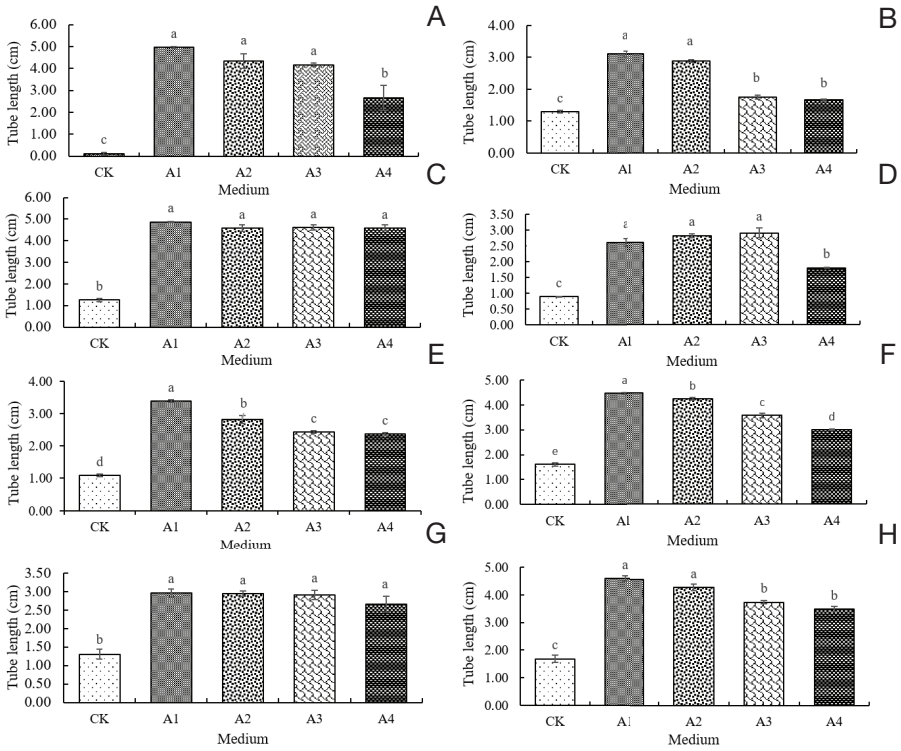

Figure 4. Pollen tube lengths of eight gesneriad taxa in different germination media. (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Saintpaulia* 'Lyon's Party Parasol', (F) *Sinningia* 'HCY's Peach Fragrance', (G) *Streptocarpus saxorum*, and (H) *Smithiantha* 'An's Hanabi'. Medium specification (CK: 10S-0B-0C; A1: 10S-50B-200C; A2: 10S-50B-400C; A3: 10S-100B-200C; A4: 10S-100B-400C; S: sucrose; B: boric acid; C: calcium nitrate). Means with a common lowercase letter are not significantly different (Duncan test, $*p < 0.05$).

lengths. The medium A1 showing the highest pollen germination percentages (from 87% in *D. eberhardtii* to 95% in *Episcia* 'Thad's Yellow Bird' and *Smithiantha* 'An's Hanabi') and pollen tube lengths (from 2.60 cm in *Kohleria* 'Brimstone' to 4.96 cm in *Alsobia* 'Cygnet') was selected for screening pollen viability throughout the flowering period.

The percentages of pollen germination at each day during the flowering period of eight gesneriad taxa are shown in Figure 5. The flowering duration varied from 10 days in *Alsobia* 'Cygnet' to 18 days in *S. saxorum*. In general, the pollen germination percentages were the highest at anthesis, and then decreased as the flower aged. While in *Episcia* 'Thad's Yellow Bird', *Kohleria* 'Brimstone', *Saintpaulia* 'Lyon's Party Parasol', *Sinningia* 'HCY's Peach Fragrance', and *Smithiantha* 'An's Hanabi' the pollen grains had completely lost viability 2–5 days before the flowers withered, a slightly contrasting situation was observed in the case of *Alsobia* 'Cygnet', *D. eberhardtii*, and *S. saxorum*, where a small portion of pollen grains remained viable by the end of the flowering period.

The speed at which the germination rates of pollen grains decreased varied from plant to plant. High pollen germination percentages were recorded for only 1 day of the flowering period in *Alsobia* 'Cygnet' (89%), *Episcia* 'Thad's Yellow Bird' (97%), and *Kohleria* 'Brimstone' (94%). High percentages of pollen germination were maintained for approximately 3 days following anthesis in *Saintpaulia* 'Lyon's Party Parasol' (88%–90%), *S. saxorum* (92%–93%), and *Smithiantha* 'An's Hanabi' (93%–95%). In *Sinningia* 'HCY's Peach Fragrance', maximum pollen germination rates were recorded for 4 consecutive days (88%–92%). *Deinostigma eberhardtii* was the only plant that had high pollen germination percentages (92%–89%) lasting for 9 days of the flowering period. The reduction of the germination potential of pollen grains was more rapid in *Alsobia* 'Cygnet', *Saintpaulia* 'Lyon's Party Parasol', and *Smithiantha* 'An's Hanabi', and was more moderate in the remaining taxa.

The pollen tube lengths of eight gesneriad taxa during each day of the flowering period are shown in Figure 6. It can be seen that the pollen tube length was greatly affected by the time of pollen collection. The longest pollen tubes were found at the first day of flower opening in *D. eberhardtii* (3.48 cm), *Episcia* 'Thad's Yellow Bird' (4.20 cm), *Saintpaulia* 'Lyon's Party Parasol' (3.35 cm), *Sinningia* 'HCY's Peach Fragrance' (4.37 cm), *S. saxorum* (3.88 cm), and *Smithiantha* 'An's Hanabi' (4.61 cm). In *Kohleria* 'Brimstone', the longest pollen tubes were recorded for 2 days after flower opening (4.04–4.14 cm). The only plant with high pollen tube lengths that lasted for 3 days after flower opening

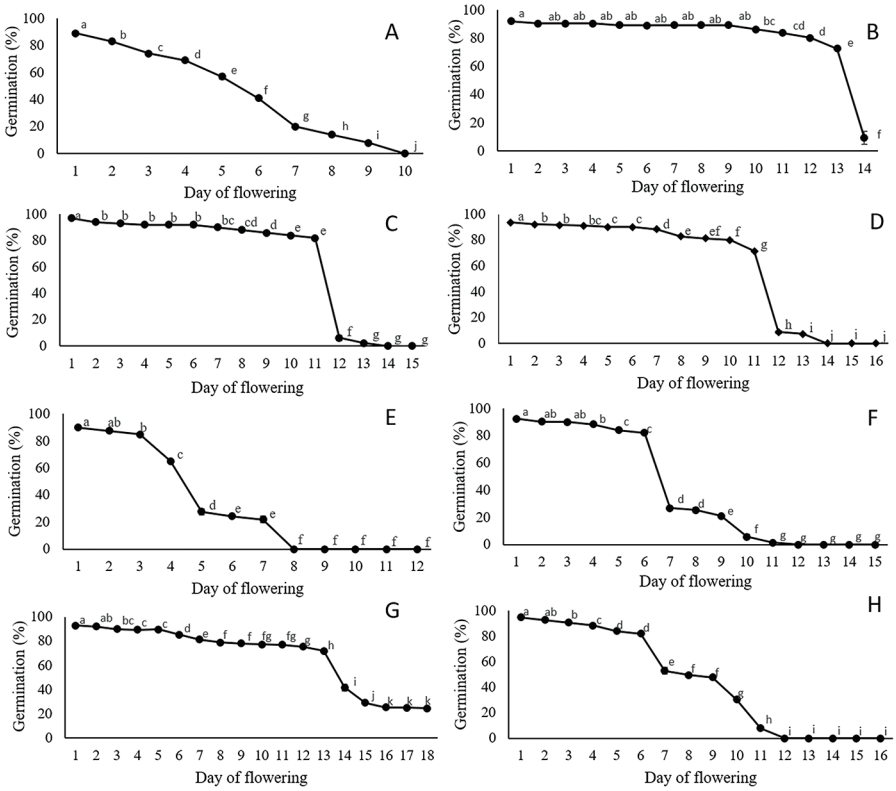

Figure 5. Daily pollen germination percentages throughout the entire flowering period of eight gesneriad taxa. (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Saintpaulia* 'Lyon's Party Parasol', (F) *Sinningia* 'HCY's Peach Fragrance', (G) *Streptocarpus saxorum*, and (H) *Smithiantha* 'An's Hanabi'. Means with a common lowercase letter are not significantly different (Duncan test, $*p < 0.05$).

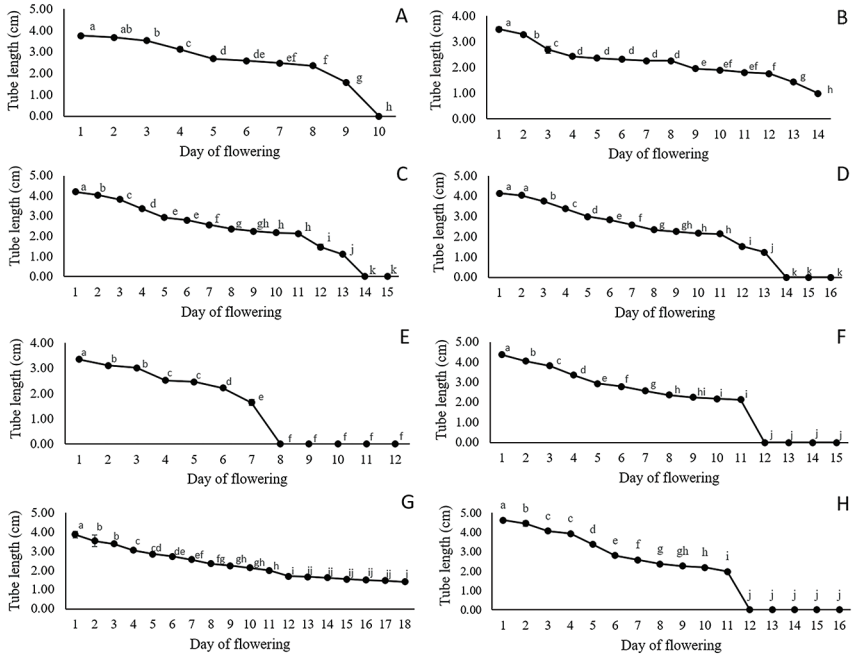

Figure 6. Daily pollen tube lengths throughout the flowering period of eight gesneriad taxa. (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Saintpaulia* 'Lyon's Party Parasol', (F) *Sinningia* 'HCY's Peach Fragrance', (G) *Streptocarpus saxorum*, and (H) *Smithiantha* 'An's Hanabi'. Means with a common lowercase letter are not significantly different (Duncan test, $*p < 0.05$).

was *Alsobia* 'Cygnet' (3.52–3.75 cm). In all genera, the pollen tube lengths decreased rapidly throughout the flowering period.

Evaluation of stigma receptivity

Morphological observations of eight gesneriad taxa revealed wet stigmas in *S. saxorum* and *Saintpaulia* 'Lyon's Party Parasol', and dry stigmas in the remaining taxa. The dry stigmas had a hydrated cuticular layer or pellicle at maturity but no free-flowing secretion. The dry stigma is clear white and slightly larger than the style at anthesis. The stigmatic surface was closed with unicellular papilla during the first days of flowering. At Day 4, the stigma lobe was differently enlarged and showed a small opening. At the completion of 8–10 days after anthesis, the stigma cavity was clearly visible to the naked eye for the various species tested (Figure 7). At 11–13 days, the papillae were collapsed at stigmatic tissue in an older stigma, and the cells missed turgidity. After 14 days, the stigmatoid tissue was completely degenerated. For the wet stigma, secretion was already observable at Day 1 of flowering. The secretion was produced by the stigmatoid tissue located under the receptive papillae surface. The deficiency of papillae turgidity is preceded by the beginning of exudate production. The stigmas and papillae were turgid by the fourth day after flowering (Figure 7).

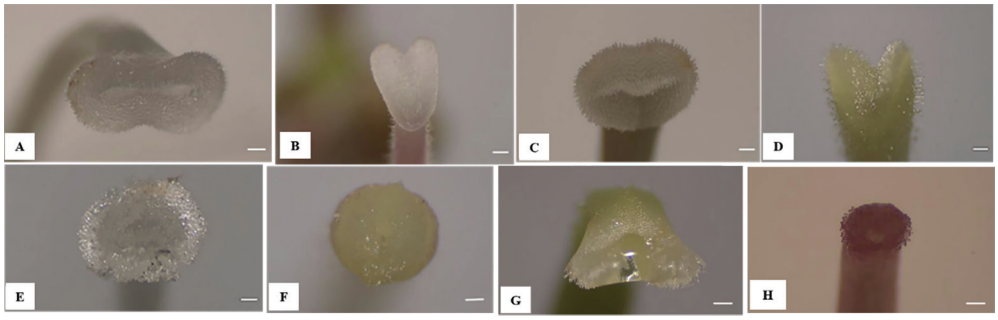

Figure 7. Stigma morphology of eight gesneriad taxa. Dry stigmas of (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Sinningia* 'Mark Twain', and (F) *Smithiantha* 'An's Hanabi' at Day 8–10 of flowering. Wet stigmas of (G) *Streptocarpus saxorum* and (H) *Saintpaulia* 'Lyon's Party Parasol' at Day 1 of flowering. Bars = 1 cm.

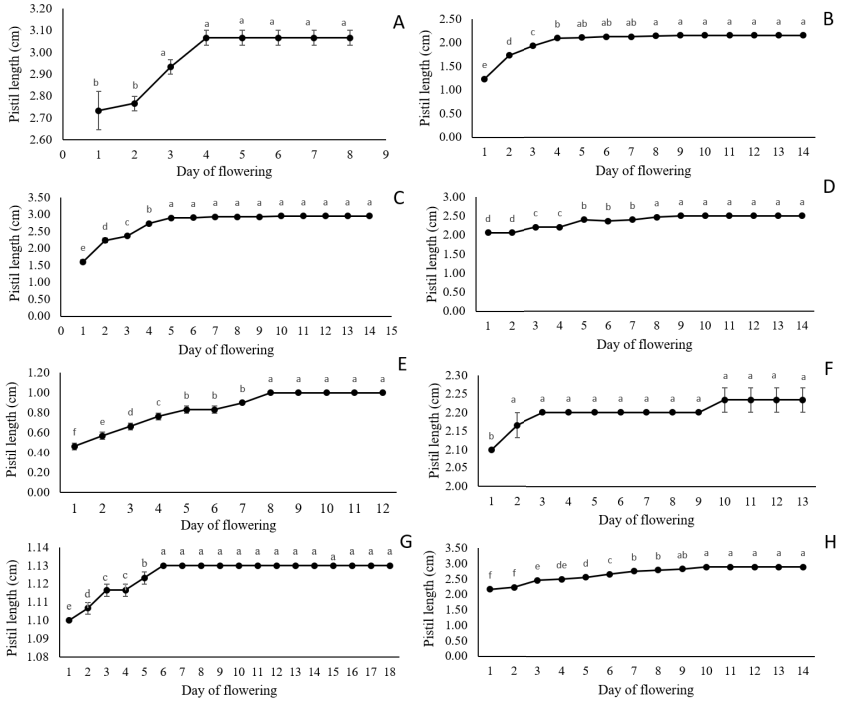

Figure 8. Daily pistil lengths throughout the flowering period of eight gesneriad taxa. (A) *Alsobia* 'Cygnet', (B) *Deinostigma eberhardtii*, (C) *Episcia* 'Thad's Yellow Bird', (D) *Kohleria* 'Brimstone', (E) *Saintpaulia* 'Lyon's Party Parasol', (F) *Sinningia* 'Mark Twain', (G) *Streptocarpus saxorum*, and (H) *Smithiantha* 'An's Hanabi'. Means with a common lowercase letter are not significantly different (Duncan test, $*p < 0.05$).

The length of the pistils in all the species tested increased progressively from Day 1 until it reached the maximum at Day 3 for *Sinningia* 'HCY's Peach Fragrance', Day 4 for *Alsobia* 'Cygnet' and *D. eberhardtii*, Day 5 for *Episcia* 'Thad's Yellow Bird', Day 6 for *S. saxorum*, Day 7 for *Smithiantha* 'An's Hanabi', and Day 8 for *Kohleria* 'Brimstone' and *Saintpaulia* 'Lyon's Party Parasol' (Figure 8). Thereafter, the pistil length remained constant till the end of the flowering period.

DISCUSSION

Sexual hybridisation can enrich the genetic diversity of flowering plants by creating new hybrids with combined parental characteristics. The timing of pollination is vital for successful hybridisation due to possible disparity between male and female reproductive maturity. Pollen viability and stigma receptivity are critical factors affecting the timing of pollination since they occur only for a limited time and at a specific period during flower growth (Heslop-Harrison, 2000).

The *in vitro* pollen germination test has been considered an appropriate approach to evaluate pollen viability (Sulusoglu and Cavusoglu, 2014; Gandadikusumah et al., 2017; Damayanti et al., 2021). However, the *in vitro* conditions for pollen germination vary widely among species, especially in terms of the medium requirement (Ahmad et al., 2012; Mudi and Mondal, 2014). Medium components such as boric acid, calcium nitrate, potassium nitrate, and magnesium sulphate have all been shown to have a great influence on pollen germination and pollen tube growth (Sahar and Spiegel-Roy, 1984). In this study, the *in vitro* pollen germination of different gesneriad species was conducted on media containing various amounts of sucrose, boric acid, and calcium nitrate. Supplementation with an adequate amount of sucrose to the medium is crucial for pollen germination and pollen tube development in gesneriads. In this study, higher germination percentages and growth of pollen tubes were obtained with 10% sucrose compared to 20% sucrose, whereas no germination was found with 30% sucrose. This result is in agreement with that of Fragallah et al. (2018), who found that both germination and pollen tube growth of Chinese fir was best in media containing less than 15% sucrose. According to Lin et al. (2017), both pollen germination and pollen tube elongation are hindered by high sugar concentrations. This could be due to the imbalanced osmotic pressure in the pollen grain, which inhibited pollen germination when pollen was immersed in media with high sucrose concentrations. Boron is well known for its function in pollen germination and pollen tube development by reducing pollen bursting (Patel and Mankad, 2014). Pollen germination is generally hampered by a lack of boric acid, which has been demonstrated in a variety of plant species (Ahmad et al., 2012; Liu et al., 2013; Kavand et al., 2014). The results of this study showed that boric acid at 50 mg · L^{-1} or 100 mg · L^{-1} enhanced pollen germination and pollen tube growth compared to media devoid of boric acid. The concentrations of boric acid used in this study were coherent with those used by Wang et al. (2003) and Acar et al. (2010), where 100 mg · L^{-1} boric acid enabled germination of pollen grains and growth of pollen tubes in *Pistacia*, while higher concentrations (2,000 mg · L^{-1}) hindered pollen germination and growth of pollen tubes. Calcium plays a role in cation balance and is required for the formation of pollen tubes (Jayaprakash, 2018). Extracellular calcium has been shown to be essential for pollen tip development (Steinhorst and Kudla, 2013). Dickinson (1967) showed that calcium regulates the permeability of pollen tube membranes. The absence of calcium in the medium increases membrane permeability, leading to loss of internal metabolites (Dabgar and Jain, 2001). This could explain why the pollens germinated poorly in CK medium in this study. Our results did not reveal any difference in pollen germination and pollen tube growth between 200 mg · L^{-1} and 400 mg · L^{-1} calcium nitrate. These concentrations are comparable to those used by Steinhorst and Kudla (2013), who stated that the dicotyledonous plants required between 300 mg · L^{-1} and 400 mg · L^{-1} calcium nitrate for pollen germination and pollen tube growth. Also, Tuinstra and Wedel (2000) showed that 300 mg · L^{-1} calcium nitrate was adequate for pollen germination as well as pollen tube development in sorghum. In this study, the medium A1, which contained a combination of 10% sucrose, 50 mg · L^{-1} boric acid, and 200 mg · L^{-1} calcium nitrate, proved to be the best for pollen germination and pollen tube growth in all taxa tested; accordingly, the same was selected for the evaluation of pollen viability throughout the flowering period.

Understanding when pollen grains possess high viability during the flowering period is essential prior to any cross-pollination attempt. When hybridisation works are undertaken, it is important that the pollen grains are collected at an appropriate stage of maturation to ensure pollination success. In this study, the pollen grains were screened for viability at each day of the flowering period, which lasted from 10 days to 18 days depending on the plant tested. The results indicated that the pollen grains were fully mature at the time of flower opening and showed maximum germination and pollen tube growth at anthesis. Thereafter, the pollen grains gradually decreased in viability with advancing flower age, although the decline rate varied between examined plants. According to Ferri et al. (2008), the quick decline in pollen viability could be related to the lack of gut water permeability, leading to a decrease in pollen hydration. This pattern was also observed in *Olea europaea* 'Picual' (Aguilera and Valenzuela, 2013). In this study, the best time to collect pollen grains depended on the rate of pollen viability degradation, which was largely species-dependent. Pollen grains of *Alsobia* 'Cygnet', *Episcia* 'Thad's Yellow Bird', and *Kohleria* 'Brismtone'

were best collected on Day 1 of flower opening, whereas pollen grains of *D. eberhardtii* could be collected at any time between Days 1 and 9 during flowering. The other gesneriad taxa showed intermediate duration of pollen viability where pollen grains could be collected between Days 1 and 3. The same results were observed in *Aeschynanthus tricolor* whereby the highest germination percentage was discovered on the day of anthesis (96%), whereas pollen collected 5 days following anthesis had the lowest germination (5.6%) (Gandadikusumah et al., 2017). It was suggested by Shivanna and Rangaswamy (1992) that genotypic differences among plants are one of the variables affecting pollen viability. In this study, only few taxa had viable pollen grains at the end of the flowering period, and most of the taxa lost pollen viability completely a few days before the end of the flowering period.

In plants, stigma types have been classified as either dry or wet, with the former having a proteinaceous extracellular pellicle layer overlying the stigma surface and generally lacking any free-flowing secretion, whereas the latter is characterised by a fluid secretion at the receptive state (Heslop-Harrison and Shivanna, 1977). In gesneriads, the stigmas can be of wet or dry type, and 1–2 lobed, with usually unicellular papillae (Watson and Dallwitz, 1991). In this study, the gynoecium of *Alsobia* 'Cygnet', *Episcia* 'Thad's Yellow Bird', *D. eberhardtii*, *Smithinatha* 'An's Hanabi', *Sinningia* 'Mark Twain', and *Kohleria* 'Brimstone' were revealed as having dry stigmas, whereas *S. saxorum* and *Saintpaulia* 'Lyon's Party Parasol' presented wet stigmas. Wet stigmas have resulted in the development of exudates high in proteins, carbohydrates, free amino acids, and lipids, which created an ideal environment for hydration, germination, and early tube formation of the pollen. In *Sanango racemosum*, the stigmatic cells secrete a viscous substance underneath the cuticle, and the pollen tubes penetrate the cuticle and grow towards the base of the papilla in the space thus generated (Maldonado and Otegui, 1997).

At certain stages of the floral life cycle, stigma receptivity may have a considerable impact on the percentage of pollination success (Galen et al., 1987). As a result, testing the timing and duration of stigma receptivity should be included in breeding trials or pollination operations (Stone et al., 1995). In this study, the influence of flower age on pistil length of different gesneriad taxa was investigated. The results showed that the pistil length gradually increased after anthesis and reached the maximum from Days 3 to 8 after anthesis depending on the taxon evaluated. The pistils of *Sinningia* 'Mark Twain' reached maximum length as early as 3 days after anthesis, whereas those of *Kohleria* 'Brimstone' and *Saintpaulia* 'Lyon's Party Parasol' took as long as 8 days to reach maximum length, indicating a slower maturation of pistils in these plants. Variation in the speed of pistil maturation and length of a fully mature pistil was found among different gesneriad taxa, suggesting that such variation may have taxonomic value. Our result is in agreement with those of Guo and Wang (2014), who reported that the pistils of *Oreocharis pumila* reached maximum length at Day 3 of anthesis (ca. 18 mm) and lasted till Day 6, a period during which 100% stigma receptivity was recorded. It can be suggested that the pistil length can be used as an indicator of stigma receptivity and the flowers have the greatest reproductive potential towards the mid and end of the flowering period in generiads.

CONCLUSIONS

The *in vitro* pollen germination of gesneriads performed the best on a medium supplemented with 10% sucrose, 50 mg · L^{-1} boric acid, and 200 mg · L^{-1} calcium nitrate. Screening of pollen viability throughout the flowering period revealed that pollen grains showed the highest germination percentages and pollen tube lengths at the first day of flower opening in all the gesneriad taxa tested. Old stigma seemed to be more receptive than young stigma and pollen grains should be transferred to 3–8 days-old stigmas depending on the plant considered. The findings in this study suggest that higher success rates of pollination may be obtained using pollen grains collected from newly opened flowers and stigmas of mature flowers in this family.

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AUTHOR CONTRIBUTIONS

J.Y.F. designed and supervised the experiments, performed the critical revision of the figures and manuscript text. F.J.B. performed the experiments, figures plotting, statistical analysis of data and drafted the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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